Tenth Quarterly Report

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Project Title: Effect of Concentration and Temperature of Ethanol in Fuel Blends on Microbial and Stress Corrosion Cracking of High-Strength Steels

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For quarterly period ending: *December 14. 2010,*

CONTENTS

Contents 2

Table of Figures 3

Technical Status 4

Field Assessment and Microbiological Characterization 4

Laboratory Microbiological Cultivation Efforts 4

Multi-specimen Bend Testing of ASTM A-36 Carbon Steel in Ethanol/Water Solutions 6

Results and Discussion: 7

Summary: 9

Immersion Testing of API X-52 Steel Coupons in Ethanol and Water Solutions with 50g/L Acetic Acid 10

Results and Discussion: 11

Summary: 12

Electrochemical Experiments 12

Fatigue Crack Growth Rate Testing 14

Results and Conclusions: 16

Issues, Problems or Challenges: 17

Plans for Future Activity: 18

Bibliography 19

TABLE OF FIGURES

Figure 1: Microbes isolated from a fuel grade ethanol filter. A) Spore-forming microbes identified as *Paenibacillus* sp. The ability of these microbes to oxidize manganese is under investigation. B) Isolates identified as *Arthrobacter* sp. 6

Figure 2 MSBT specimen finite element model showing material stress-state 7

Figure 3 Tests 1-6 (top to bottom in legend) determined by SEM at 100x magnification. Solid markers represent coupons polished with 600-grit paper and line markers represent coupons polished with 120-grit paper. 8

Figure 4 Tests 4- 6 (top to bottom in legend) determined by stereo at 7x magnification (pitting clusters). Solid markers represent coupons polished with 600-grit paper and line markers represent coupons polished with 120-grit paper. 8

Figure 5 Pitting and corresponding crack initiation of ASTM-A36 steel after undergoing 100,000 loading cycles with a maximum stress of 75 pct. of yield strength and a stress ratio of 0.5 9

Figure 6 Localized corrosion of API X-52 pipeline steel immersed in ethanol and water solution containing 50 g/L acetic acid 11

Figure 7 API X52 steel coupons exhibiting localized corrosion and crack-like features after exposure to water and ethanol solution containing 50g/L acetic acid. 11

Figure 8 A36 Steel specimens with the biofilm after 15 days of immersion in the test solution containing acetobacter aceti 13

Figure 9 Open circuit potential as a function of time for A36 steel in the presence (AA) and absence (control) of bacteria 13

Figure 10 pH as a function of time for A36 steel in the presence (AA) and absence (control) of bacteria 14

Figure 11 Schematic results showing constant force amplitude test versus constant ΔK tests being performed for this work. 15

TECHNICAL STATUS

Technical efforts for the 9th quarter have included field sampling of fuels from ethanol fuel blend (EFB) infrastructure, laboratory cultivation experiments, MSBT experiments, immersion testing, electrochemical experiments, and FCGR equipment troubleshooting.

Field Assessment and Microbiological Characterization

Analysis of samples collected from fuel grade ethanol (FGE) production facilities and fueling terminals has indicated that microbes are present inside tanks containing FGE and ethanol fuel blends. To further evaluate the microbial diversity associated with ethanol fuel blend environments, fuel samples from fueling stations have been collected. One-liter fuel samples were collected aseptically and filtered through a 0.2 micron filter to collect any microbes (and solids) in the fuel. The filters were then used to inoculate microbial culture media. Initial results indicate that viable microbes are present in these samples. The filters were also used for DNA extractions. The extracted DNA is currently being used for 16S rRNA gene sequencing to identify the microbes present in these samples. These experiments will provide further information about the types of microbes present in fuel grade ethanol and ethanol fuel blend environments and how these microbes may impact corrosion and cracking. To date, samples have been collected from points throughout the FGE industry, including at production facilities, fueling terminals and fueling stations. To our knowledge, this is the first such survey of the FGE industry.

Laboratory Microbiological Cultivation Efforts

A number of microbes that may potentially increase the corrosion of metals have been identified via 16S rRNA gene sequencing in samples collected from ethanol industry infrastructure. Cultivation experiments have been designed to isolate and grow these potentially corrosive microbes in the laboratory. A variety of microbial growth media have been inoculated with samples collected from ethanol industry infrastructure. *Viable cells have now been obtained from all samples, including samples collected from environments containing FGE with water concentrations under 1 pct.* These experiments indicate that viable microbes with potentially corrosive metabolisms are present in many locations along FGE storage and transportation systems.

Recent cultivation experiments have included the isolation of microbes from a FGE filter sample. A small portion of a FGE filter was collected aseptically and used to inoculate microbial growth media. The isolates were identified by 16S rRNA gene sequencing. Preliminary analysis indicates that spore-forming isolates are *Paenibacillus* sp. (Figure 1-A). These isolates as well as other spore-forming isolates are being examined to determine if they promote the oxidation of manganese, a component of steel. Enzymes present on the spores of some microbes are capable of promoting the oxidation of manganese. Microbial oxidation of metal species may result in increased corrosion and cracking. Isolates identified as *Arthrobacter* sp. have also been cultivated from the FGE filter (Figure 1-B). *Arthrobacter* sp. are common in soils and are quite resistant to dessication, though they do not form spores (Madigan, et al. 2009). *Arthrobacter* sp. are known to degrade hydrocarbons (Efroymson and Martin 1991), which could result in degraded fuels, and these types of microorganisms have been found in aviation fuel tanks (Rauch, et al. 2005). These microbes are also known to reduce iron species (Fe(III) reduced to Fe(II)). Microbial iron reduction has been implicated in MIC, though the literature contains conflicting reports on the impact of iron-reducing microbes on corrosion (Lee and Newman 2003). Further tests will be conducted to determine how these microbes impact corrosion and cracking of steels exposed to ethanolic environments.

Previous cultivation experiments included the cultivation and identification of acetic acid producing bacteria (*Acetobacter* sp.) isolated from FGE spillage containment tanks. These sorts of tanks were recently discovered to have experienced internal corrosion issues, and some of the tanks have been reported to smell like vinegar. *Acetobacter* sp. are known to oxidize ethanol to acetic acid. On-going experiments are being conducted to determine the ability of these microbes to survive high ethanol concentrations and exposure to ethanol fuel blends. These acetic acid producing microbes are also being used in electrochemical experiments to determine how these microbes affect the corrosion of steel in ethanolic environments.

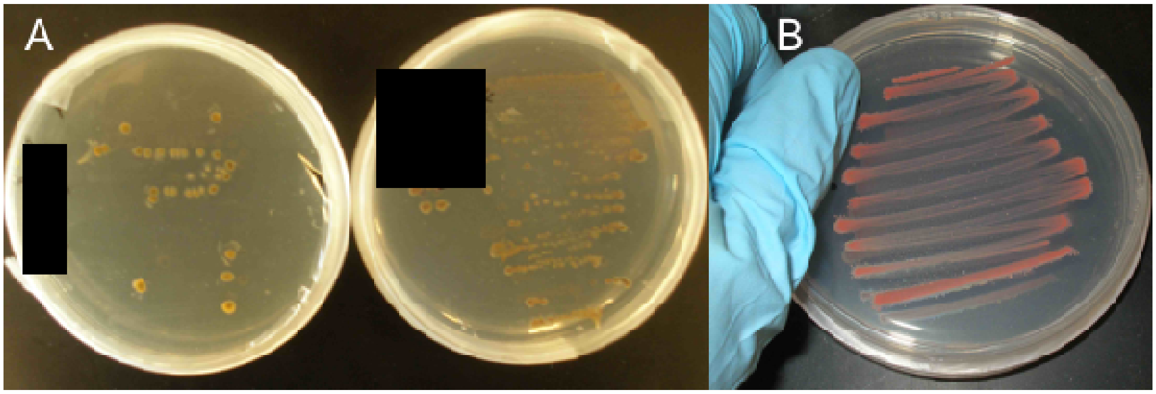


Figure 1: Microbes isolated from a fuel grade ethanol filter. A) Spore-forming microbes identified as *Paenibacillus* sp. The ability of these microbes to oxidize manganese is under investigation. B) Isolates identified as *Arthrobacter* sp.

Multi-specimen Bend Testing of ASTM A-36 Carbon Steel in Ethanol/Water Solutions

Testing was conducted to evaluate the effect of cyclic loading on pitting and crack initiation of steel in ethanol and water solutions

ASTM A-36 steel specimens were machined and polished with 600 (tests 1-3) and 120 (tests 4-6) grit papers. The coupons were cleaned, rinsed with DI water and acetone, and stored in a dessicator. A solution containing 93 vol. pct. ethanol, 7 vol. pct. DI water, and 8 ppm chloride was prepared. The specimen and solution were introduced into a chamber of the MSBT system in the Fuels Testing Laboratory at National Institute of Standards and Technology (NIST) in Boulder, CO. The specimens were loaded for 25,000, 50,000 and 100,000 cycles over 3 to 12 days. Testing conditions for the experiments are described in Table 1

Table 1 Testing duration and loading criteria for MSBT experiments

Test [#]	Time [days]	Loading [Cycles]	Maximum Stress [% Yield Stress]	Stress Ratio [min/max]	Frequency [Hz]
1	3	25 k	75	0.5	0.1
2	8	50 k	75	0.5	0.1
3	14	100 k	75	0.5	0.1
4	12	100 k	75	0.5	0.1
5	12	100 k	100	0.5	0.1
6	12	100 k	100	0.5	0.1

Cyclic loading was performed to a maximum load corresponding to 75 pct. of the yield stress for the steel with a stress ratio of 0.5. Following cyclic loading, the specimens were cleaned and all surface scale was removed. Surface imaging was performed using light microscopy, stereomicroscopy and scanning electron microscopy (SEM) to evaluate the morphology of the surface corrosion. Pitting density was measured in the areas of the stressed zone (SZ) and the un-stressed zone (USZ) of the coupon as depicted in Figure 2. The outer fiber of the SZ on the coupon experienced cyclic loading as described above. The USZ theoretically experienced no stress when loaded under four-point bending.

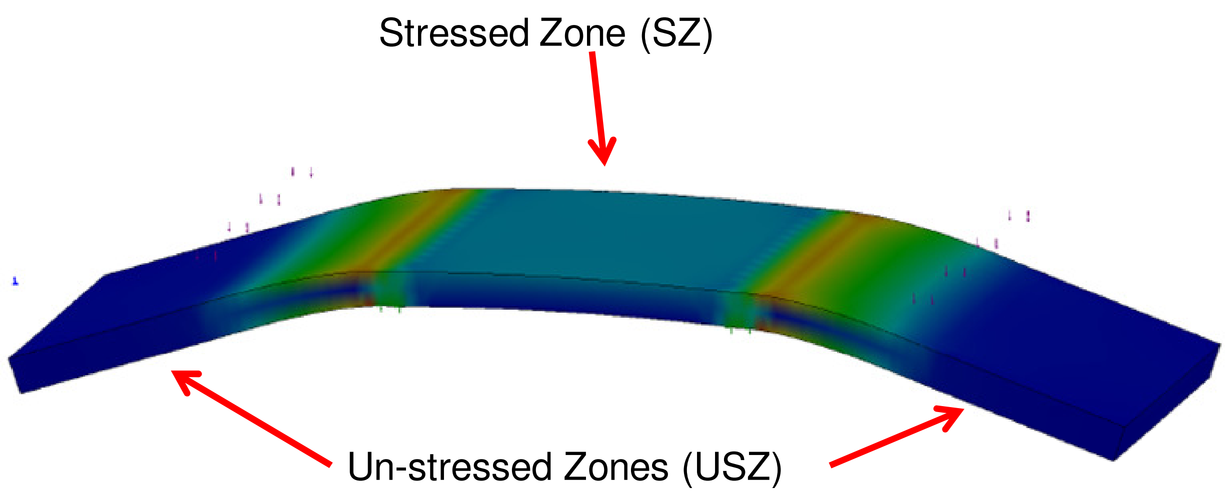


Figure 2 MSBT specimen finite element model showing material stress-state

Results and Discussion:

Extensive pitting was observed in the SZ and USZ of the steel surface. Figure 3 shows pitting densities for Tests 1 through 6 measured by SEM at 100x magnification. It can be observed that pitting density was generally greater for the SZs of the coupons when compared with the USZs. The most evident and repeatable example of this occurred with duplicate Tests 5 and 6. Pitting densities in these cases increased by nearly 50 pct. Figure 4 shows pitting densities for Tests 4 through 6 measured by stereomicroscopy at 7x magnification. Pitting events observed at this magnification most commonly consisted of several pits growing together in a cluster. As was observed with the pitting densities at 100x, the density of these relatively larger pitting clusters was observed to be greater in the SZ of the coupon when compared to the USZ.

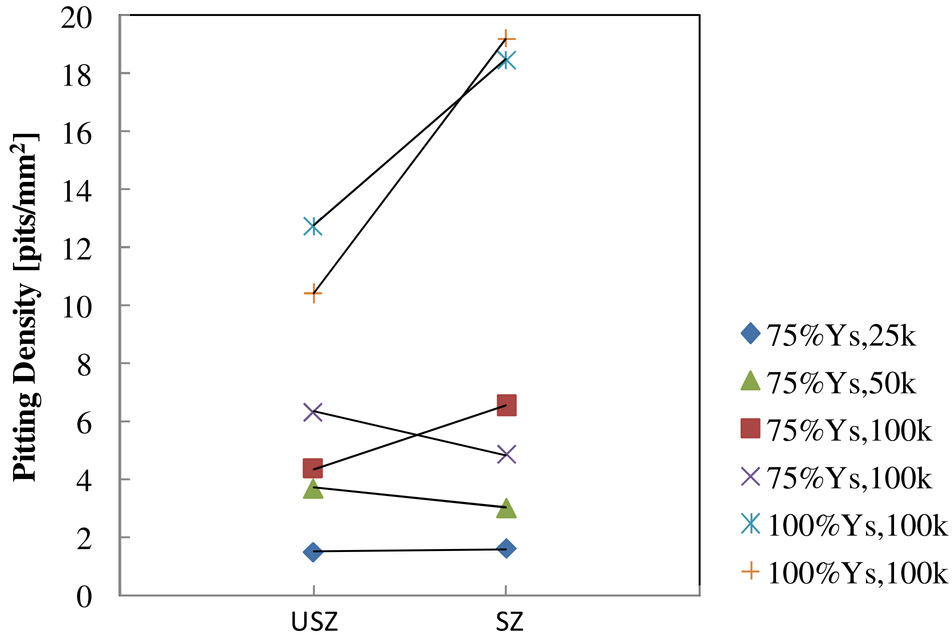


Figure 3 Tests 1-6 (top to bottom in legend) determined by SEM at 100x magnification. Solid markers represent coupons polished with 600-grit paper and line markers represent coupons polished with 120-grit paper.

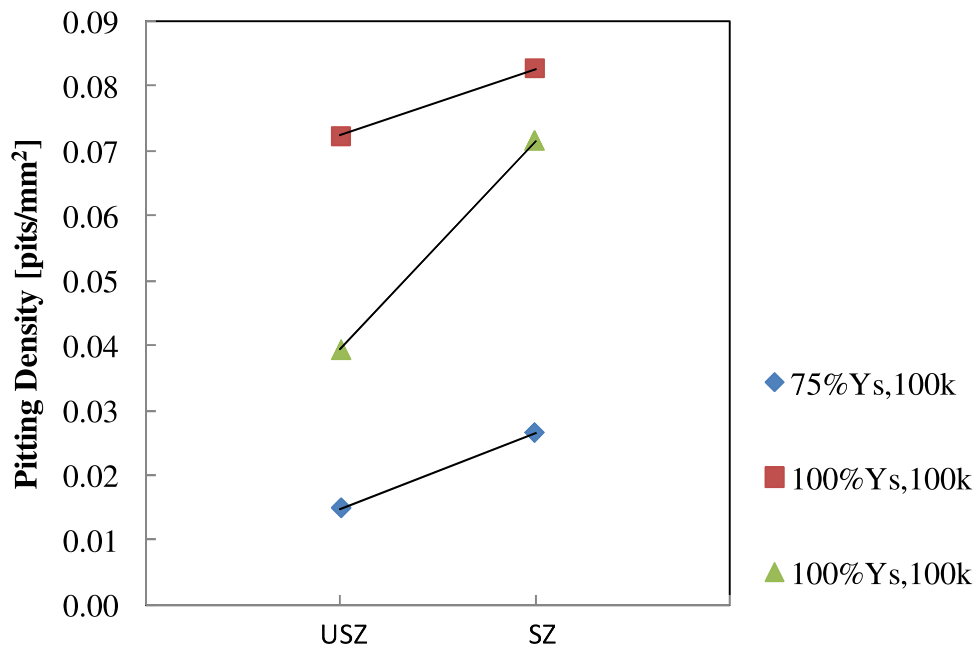


Figure 4 Tests 4- 6 (top to bottom in legend) determined by stereo at 7x magnification (pitting clusters). Solid markers represent coupons polished with 600-grit paper and line markers represent coupons polished with 120-grit paper.

Pits on the steel surface experiencing cyclic stresses (below the yield stress) appeared to have cracks initiating from the edge of the pits and propagating across the steel surface often times perpendicular to the applied stress. In Figure 5, light microscopy of the steel surface shows extensive cracking initiating from sites along the pit edges.

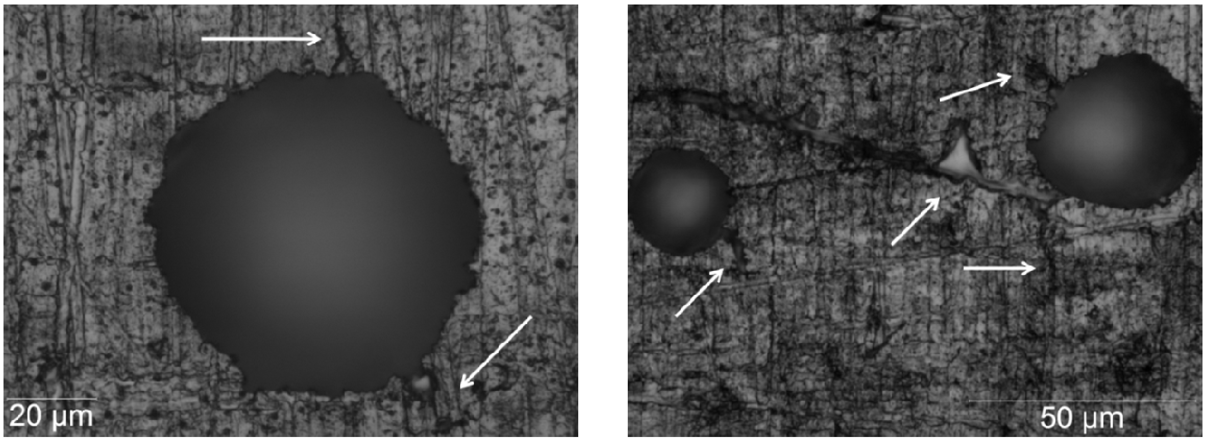


Figure 5 Pitting and corresponding crack initiation of ASTM-A36 steel after undergoing 100,000 loading cycles with a maximum stress of 75 pct. of yield strength and a stress ratio of 0.5

Summary:

1) Pitting densities measured by stereomicroscopy at 7x magnification and by SEM at 100x magnification were generally greater for the SZ of the coupons when compared to the USZ of the coupons.

2) The observed surface cracks could be cracks that were initiated by either the stress concentration in the pits during fatigue loading, corrosion fatigue, or by ethanol stress corrosion cracking (eSCC). Recent evaluations of eSCC have suggested that eSCC initiation is only associated with the macroscopic yielding of the material (Lou, Yang and Singh 2009). These studies could be the first to demonstrate environmental cracking below macroscopic yield strains in FGE systems. Cross-sectional metallography and further imaging will be conducted to evaluate the mode of crack propagation. The observed mode of cracking can then be compared to the cracking mechanisms proposed above.

3) Crack initiation at these relatively lower stresses could be representative of conditions experienced by steel pipelines. If similar conditions were experienced, cracks such as these could have the potential to propagate, potentially causing pipeline failures. Further testing

should be undertaken to find thresholds for crack initiation in these circumstances. These thresholds should include loading parameters, such as maximum stress, stress ratio, and number of loading cycles, chemical thresholds for constituents such as water, chlorides, and oxygen, and electrochemical thresholds such as the electrochemical potential of the steel including the localized potential near the crack.

3) Finite element analysis has indicated that localized plastic strain rates on the crack walls near the mouth of the pit could be above the threshold required to initiate SCC (Turnbull, Wright and Crocker 2010). The images generated in the present study agree with the finite element modeling performed by Turnbull et al. and may serve to shed light on the mechanisms underlying the transition from pitting corrosion to environmental cracking processes such as SCC and corrosion fatigue. A more complete understanding of cracking processes could enable improved material selection as well as improved corrosion monitoring and mitigation.

Immersion Testing of API X-52 Steel Coupons in Ethanol and Water Solutions with 50g/L Acetic Acid

These tests were performed to evaluate corrosion of API 5L X-52 pipeline steel in ethanol/water solutions with concentrations of acetic acid (produced by acetic acid bacteria) similar to those isolated from a FGE blending terminal (a tank failure at this terminal is suspected to have occurred due to microbial acetic acid production).

The X-52 steel coupons were machined and polished with 120-grit paper. The coupons were cleaned, rinsed with DI water and acetone, and stored in a dessicator. A solution containing 85 wt. pct. creek water, 10 wt. pct. ethanol, and 5 wt. pct. acetic acid was prepared. This concentration of acetic acid is known to be able to be produced by acetic acid bacteria similar to those isolated and sequenced in the ethanol spillage tank (Lu, Lee and Chen 1999). A steel specimen and solution were introduced into a test cell in the Corrosion Laboratory at Colorado School of Mines (CSM) in Golden, CO. The specimen was immersed for 15 days. The specimen was removed and rinsed with DI water. All scale was removed prior to inspection and imaging. Surface imaging was performed using light microscopy to evaluate the morphology of the surface corrosion.

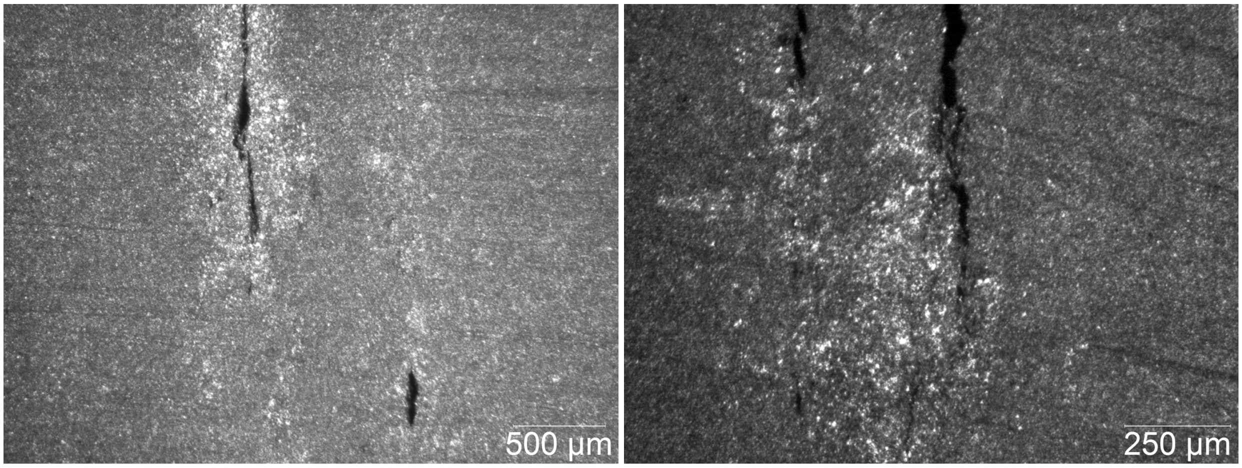
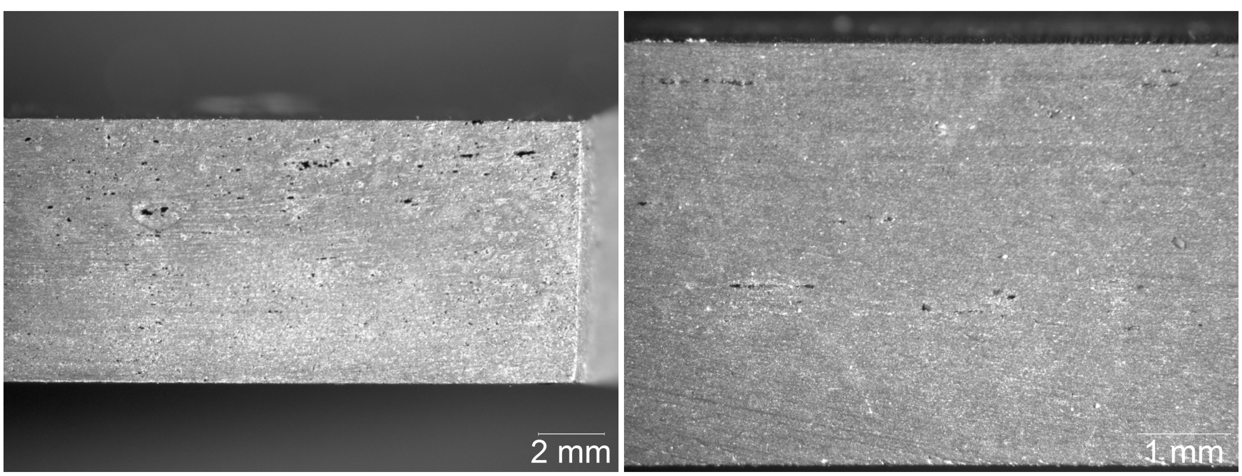


Figure 6 Localized corrosion of API X-52 pipeline steel immersed in ethanol and water solution containing 50 g/L acetic acid

Results and Discussion:

Extensive damage to the surface of the coupons was observed as shown in Figure 7. Localized corrosion was observed in a single orientation along the surface of the steel coupons. This preferential orientation was consistent when viewing different surfaces on the rectangular coupon. The end view of the coupon, seen in Figure 7a, shows the corrosion tunnels or crack-like features exiting the coupon. The side view of the coupon, seen in Figure 7b shows the corrosion tunnels again running parallel to the top view of the coupon shown in Figure 6.



(a) (b)

Figure 7 API X52 steel coupons exhibiting localized corrosion and crack-like features after exposure to water and ethanol solution containing 50g/L acetic acid.

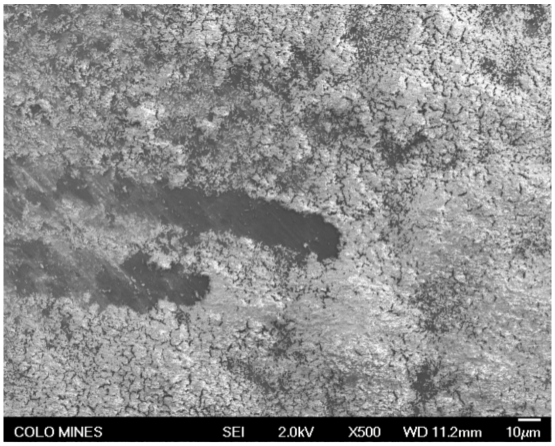
Summary:

Microbiologically Influenced Corrosion (MIC) by acid producing bacteria (APB) could compromise the integrity of steel pipelines by causing localized corrosion and initiation of cracking. MIC by microbes producing acetic acid has been documented to form corrosion "tunnels" in the rolling direction of pipeline steel and associated with inclusion, specifically MnS (Pope, et al. 1988). Localized corrosion along sulfide inclusions is commonly observed in older steels in strong acid environments. Cross-sectional metallography will be applied to determine the morphology of the corrosion and look for any evidence of cracking associated with the corrosion sites. If MIC occurs during suspension of normal pipeline operation, and FGE is subsequently resumed, MIC damage retained in the pipeline could potentially serve as sites of eSCC initiation. MIC by APB should be further studied to evaluate the possible decline in integrity of pipelines experiencing these conditions. Mechanical testing methods such as low cycle bend testing (below yield strain), impact testing, and fatigue crack growth rate (FCGR) testing could be performed to measure changes in mechanical integrity caused by MIC in fuel ethanol environments.

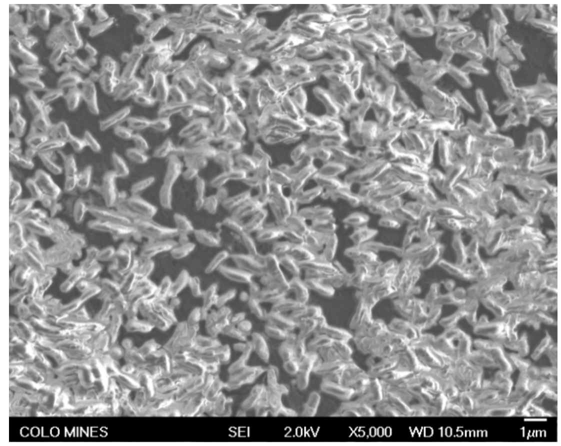
Electrochemical Experiments

Electrochemical experiments to investigate the role of acetic acid producing bacteria on the corrosion behavior of A36 mild carbon steel were performed and the analysis is in progress. Specimens were also being investigated in the control solution in order to compare the behavior of steel in the presence and absence of bacteria. The composition of the control solution taken at the start of the experiment comprised of 5 volume percent ethanol in de-ionized water and culture media. The solution containing bacteria comprised of the control solution inoculated with the bacterial culture. Immersion testing experiments were run in parallel to understand the morphology of the steel specimens both with the biofilm and after removing the biofilm to see the presence of pits if any.

Figure 1 shows the steel specimens covered with the biofilm after 15 days of immersion in the test solution, both of low and high magnifications. Rod shaped colonies of acetobacter aceti (AA) covering majority of the metal surface can be seen in the figure.



Biofilm covering the surface of the specimen



Bacterial colony forming the biofilm at high magnification

Figure 8 A36 Steel specimens with the biofilm after 15 days of immersion in the test solution containing acetobacter aceti

Figure 2 shows open circuit potential (OCP) as a function of time for A36 steel in the presence and absence of bacteria. The bacteria appear to have produced ennoblement in OCP values of steel as can be observed in Figure 2. The cause for this behavior is presently being explored as a part of the detailed analysis for this investigation.

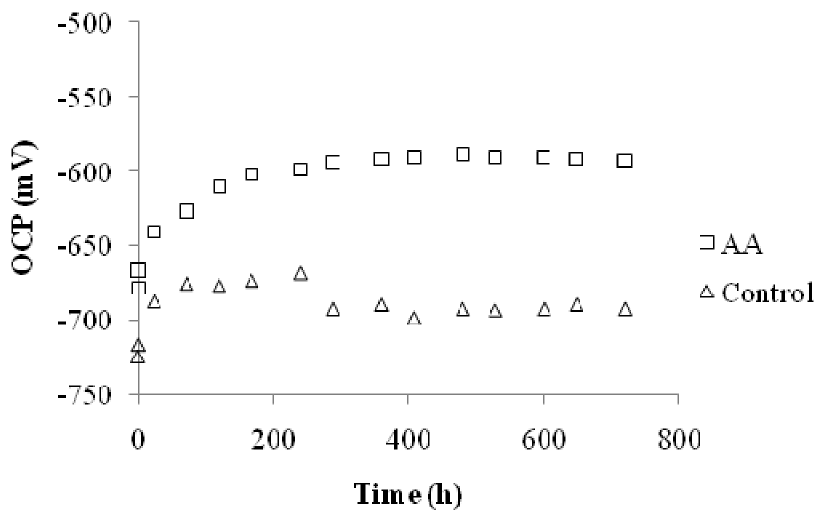


Figure 9 Open circuit potential as a function of time for A36 steel in the presence (AA) and absence (control) of bacteria

Figure 3 shows pH as a function of time for A36 steel in the presence and absence of bacteria. The pH values of the control solution remain constant at a near-neutral value for the entire duration of the experiments. The pH values of the solution containing acetobacter aceti are lower than the control due to the formation of acetic acid by bacteria. The pH decreases up to around 250 hours following which it remains more or less at the same value. This might be due to the growth and the metabolic activity of bacteria which appears to be the highest during the initial time duration.

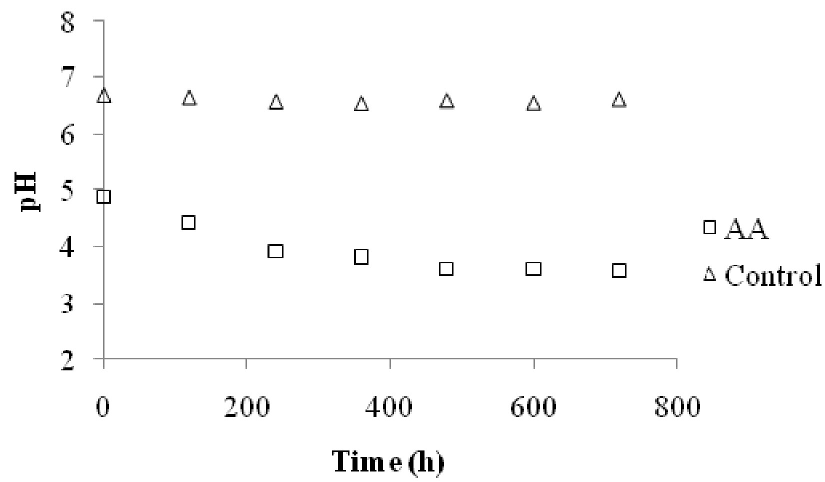


Figure 10 pH as a function of time for A36 steel in the presence (AA) and absence (control) of bacteria

Fatigue Crack Growth Rate Testing

The test machine controller was updated to a newer model (MTS FlexTest GT) recently and the older versions of fatigue software were not compatible with the new controller. This was unexpected and required an upgrade in controller software. The new software was acquired recently and has been installed. This software has the complete capability necessary to finish the remainder of the proposed fatigue work for this project.

Compact tension C(T) specimens were “pre-cracked” using the reaction frame that was fabricated and installed in the previous quarter. One specimen of each of the three different test alloys (A36, X52, X70) was pre-cracked. The pre-cracking procedure was performed to introduce a sharp crack in the C(T) specimens in preparation for the fatigue crack growth rate (FCGR) tests. Current work is focused on measuring the FCGR of the steels in air at ambient conditions. These tests will be used as a baseline for subsequent determination of FCGR in simulated environmental conditions with ethanol and microbes.

A procedure has been selected to perform the fatigue studies in a manner that will provide a significant amount of crack growth information in a shorter period of time than constant force testing. During constant force FCGR testing, the applied force amplitude ($Force_{max}$ – $Force_{min}$) is held constant throughout the test. Initially the crack growth rate is very small since the crack is short and low stress intensity amplitudes (ΔK) are present at the growing crack tip. As the crack grows, the stress intensity amplitude increases due to the decreasing specimen cross-section. For environmental FCGR tests, this can result in test durations of several weeks or more since test frequencies are typically 1Hz or less. The current work will be performed by maintaining constant stress intensity amplitude. Multiple data points will be generated to determine crack growth rate trends in the stead-state (Mode II) crack growth region. This is the region that is important to pipeline designers since they do not design for Mode I (initiation) or Mode III (mechanical overload). The difference in results between the two tests is shown schematically below. Note that the slope of the Mode II crack growth region is still easily determined, with the resulting beneficial time savings. A FCGR test of X70 is currently being conducted using this procedure. This test will be repeated on the A36 and X52 alloys in air before moving on to fuel grade ethanol and other solutions.

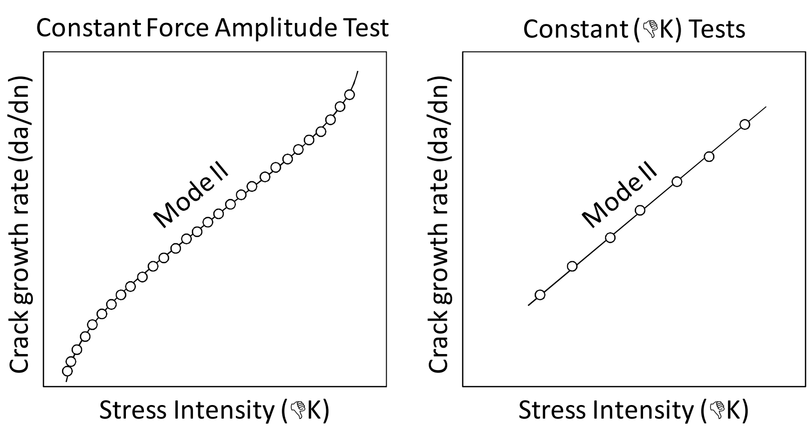


Figure 11 Schematic results showing constant force amplitude test versus constant ΔK tests being performed for this work.

RESULTS AND CONCLUSIONS:

- Analyses of samples collected from fuel grade ethanol production facilities, fueling terminals and fueling stations have continued. DNA sequencing suggests that microbes with metabolisms that may increase corrosion are present in ethanol fuel industry infrastructure.
- Microbial growth and corrosion experiments have continued to determine the ability of microbes to affect corrosion in high-ethanol environments.
- Viable microbes have been cultivated from a variety of ethanol fuel industry infrastructure samples, including samples collected from fuel grade ethanol environments containing less than one percent water. These experiments suggest that viable microbes potentially capable of increasing corrosion of steels are present in environments containing fuel grade ethanol.
- The microbial diversity associated with fuel grade ethanol and ethanol fuel blends continues to be explored. To our knowledge, limited information exists regarding the microbial diversity associated with ethanol fuel blend infrastructure.
- MIC has likely been observed on API X70 steel coupons in an E10 and water mixture using microbes acquired from active ethanol fuel infrastructure
- Acetic acid was observed to produce localized dissolution of API X50 coupons possibly due to preferential attack of the steel microstructure
- Greater pitting density was observed on stressed regions of MSBT specimens as compared to unstressed regions
- Cracking, initiating from the edges of pits, typically propagated perpendicular to the direction of principle stress in stressed regions of MSBT coupons
- Electrochemical corrosion parameters have been measured for MIC by APB of ASTM A-36 steel

ISSUES, PROBLEMS OR CHALLENGES:

- Gaining access to infrastructure for sampling purposes can be challenging

PLANS FOR FUTURE ACTIVITY:

- Continue experiments to determine the ability of microbes, including spores, to affect corrosion in high-ethanol environments.
- Pursue sampling opportunities and collect samples from EFB infrastructure.
- Continue experiments to isolate viable microbes from EFB infrastructure samples.
- Continue to evaluate the effect of cyclic stress on pitting and crack initiation in EFB environments
- Continue to test the effect of environmentally relevant cultivars and cultivars isolated from EFB infrastructure on corrosion of pipeline and tank steel using immersion specimens
- Test the effect of environmentally relevant cultivars and cultivars isolated from EFB infrastructure on corrosion of pipeline steel using bend and CT type specimens

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